

SESSION 2

BIOLOGICALS IN CROP PROTECTION

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Field performance of biopesticides

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ABSTRACT

The share of biopesticides of the total crop protection market has remained around 1%, despite repeated optimistic projections during the past decades of a much higher share. The single most important reason for this has been the difficulty in transferring the promising lab results into a consistent, reliable performance in the field. The only highly successful product group, the *Bacillus thuringiensis*-based bioinsecticides, may soon be joined by effective mycoinsecticides, viral pesticides, biofungicides, bioherbicides, and others, based on the significant advances in production, formulation and application technology, as well as in increased knowledge and appreciation of the ecological principles involved. The speed and degree of biopesticide activity can now in many cases be effectively enhanced, and the persistence and selectivity optimised so that at least some products can be expected to conquer a major share of the global pesticide markets. Many other products will occupy an increasing number of specific market niches and support a flourishing small business community.

INTRODUCTION

The idea of using of microbes to kill or to control unwanted organisms dates back well over 100 years, and during the past 30 years has been viewed as a promising alternative to using traditional chemical pesticides (Sugavanam & Xie, 1999). Projections not longer than some ten years ago estimated biopesticides to occupy 10% or even over 20% of world crop protection market by the year 2000 (e.g. Jutsum, 1988; Baker & Dunn, 1990; Meneley, 1991; Anon., 1992). Disappointingly, their global share has remained below 1% (Lisansky, 1997). Using a broad definition for biopesticides, Menn & Hall (1999) estimated that their overall market share is now 1.3%, and it is expected to grow by 10–15% per annum in contrast to 2% for chemical pesticides.

Bioinsecticides dominate biopesticide markets, generating total global annual sales of approximately US\$ 140 million (Gelernter & Lomer, 2000). The sales of *Bacillus thuringiensis* (Bt) dwarf those of any other biopesticide product, and are worth more than double of all products based on insect pathogenic fungi, nematodes, viruses and protozoans combined (Gelernter & Lomer, 2000). Nevertheless, dozens of different biopesticide organisms are or have been commercialised around the world already (e.g. Butt *et al.*, 1999; Caulder, 1999; Sugavanam & Xie, 1999).

The main reason for the slow commercial development of most biopesticides is their often poor and erratic performance under field conditions (Lisansky, 1997). Ironically, the feature that makes Bt so much more successful on a commercial basis than other

biopesticides is the non-living status of its chief active ingredient; it is not as susceptible to the same environmental pressures as living organisms during production, storage, and use (Gelernter & Lomer, 2000). In many cases, however, recent years have seen a dramatic increase in commercialisation efforts of biopesticides. This increase has been stimulated by a number of technological advances that have the potential to greatly expand the commercial feasibility of biopesticide use (Wraight & Carruthers, 1999). Ecologists warn, however, against simplistic assumptions: '...pathogens cannot simply be sprayed into the environment and be expected to perform in a similar, and reliable, manner to the chemical pesticides they are meant to replace....' (Blanford & Thomas, 2000). In their view biopesticides can attain their full potential in playing a major role in sustainable crop protection only through a far greater and more detailed understanding of the host-parasite biology and disease dynamics (Thomas, 1999).

SUCCESS STORIES

Bacillus thuringiensis-based bioinsecticides compete successfully with chemical pesticides, especially in vegetable and fruit production, but are also used extensively in other crops such as corn and cotton, as well as in forest protection and for mosquito control. In the USA, for example, over 50% of the growing areas of cabbage, celery, eggplant and raspberry were annually treated with Bt in the middle of the 1990s, while 9% of upland cotton and 1% of corn received Bt treatments (Uri, 1999). Bt-products account for almost 25% of the total insecticide use in forest systems, and provided for example about 95% control of the black arches (nun) moth (*Lymantria monacha*) in a serious outbreak over 600,000 ha in Poland in 1994 (Butt *et al.*, 1999).

Five species of fungal bioinsecticides are commercially available in Europe (Butt *et al.*, 1999) and four in the USA (Wraight & Carruthers, 1999). These still occupy only specific niche markets, but improved performance is likely to expand considerably their scope of use. One of the newest products is based on *Metarhizium anisopliae* var. *acridum*. It has been developed for the control of locusts and grasshoppers, and field trials in Africa, Europe and Australia have shown excellent potential for successful large-scale use. For example, in Benin, 90–95% mortality of the variegated grasshopper (*Zonocerus variegatus*) was obtained in several field trials (Lomer *et al.*, 1993; Douro-Kpindou *et al.*, 1995). Similarly, a 70% reduction in the densities of the grasshopper *Hieroglyphus daganensis* was obtained (Lomer *et al.*, 1997), indicating a yet higher impact on the prevention of feeding damage (cf. Thomas *et al.*, 1998). Kooyman & Godonou (1997) obtained up to 100% infection of desert locust (*Schistocerca gregaria*) under desert conditions, and field trials in Australia have also resulted in 60–90% control of target grasshoppers and locusts (Milner *et al.*, 1997).

Several insect viruses have been commercialised and are applied all around the world, but only one is approaching the extent of use many of them are believed to possess: the NPV of *Anticarsia gemmatilis* (velvetbean caterpillar), is sprayed on over 1 million ha annually in Brazil (Moscardi, 1999). Reductions in pest populations following just one spray are over 80%, and effective crop protection is obtained at a cost which is lower than the cost of chemical insecticides (Moscardi, 1999).

Entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* are mass-produced and have been commercially available for about two decades in the USA, Europe, Australia, and elsewhere. They are primarily applied on high-value crops such as greenhouse crops, strawberries, field vegetables and tree nurseries (Ehlers & Peters, 1995).

Control of plant pathogens by commercially available biofungicides has also progressed during the past 20 years. As with many other biopesticide products, only few are available worldwide. One of these is 'Mycostop', based on *Streptomyces griseoviridis*, commercialised in 1990 in Finland. Currently, it is more widely available than any other biofungicide — it is registered in 15 countries (Kemira, 1999). The product's primary targets are *Fusarium* diseases in ornamentals, vegetables, fruits and herbs, but good results are also obtained against *Phytophthora*, *Alternaria* and *Rhizoctonia* as well as suppression of *Phytophthora* and *Botrytis* diseases (Mohammadi & Lahdenperä, 1993).

A few mycoherbicides have also proven the potential of the biopesticide strategy. An interesting case in this respect is *Phytophthora palmivora*, which was used to control the weed strangler-vine (*Morrenia odorata*) in citrus orchards in the USA. The field performance of this product, however, was *too* good — only one treatment was necessary over many years, and therefore the demand for the product decreased and finally it disappeared from the market (Hokkanen, 1997; Pilgeram & Sands, 1999).

With these and many more obvious successes with biopesticides, why is it then that the expected market potential has yet to be realised? According to Törmälä (1995), far less than 5% of the initial biopesticide leads developed at the universities ever resulted in a commercially viable biopesticide. This is disappointing to the researchers as well as to the industry. Probably the main reason for this is that although the products perform nicely in the laboratory, they fail to provide unequivocal results in field trials. In the following, several of the key factors determining field performance, and progress made with them, will be discussed.

SPEED AND DEGREE OF ACTIVITY

Obviously, biopesticides will need to provide satisfactory control of the pest in order to be useful. Weighing the available alternatives for crop protection — as the end users of the products will need to do — leads often inevitably to comparisons between chemical pesticides and biopesticides, and thus to the 'chemical pesticide paradigm'; in order to be attractive, biopesticides generally need to provide effective control very quickly. This has in the vast majority of cases proven very difficult to achieve and, therefore, appears to form the major stumbling block for successful utilisation of these biological agents.

In many cases, the speed of kill of the target organism is not critical, and these do not need to be discussed here in detail. For example, in weed management or in the suppression of plant pathogens the speed of control is seldom as important as it is in the control of many insect or mite pests, where the damage often must be stopped within days or even hours after treatment. Of the bioinsecticides, only Bt products clearly meet this criterion. Entomopathogenic nematodes often get close to that (kill within 24–48 h),

whereas entomopathogenic fungi and insect viruses usually take much longer to cause mortality.

There are two possibilities of improving the situation: to find out ways of obtaining faster action with the bioagents, or to revise the way of thinking about the control process (abandon the chemical pesticide paradigm). Significant improvements have been obtained in both cases.

Faster action by biopesticides can be obtained sometimes, for example, through the selection or engineering of more virulent strains, through increasing the effective dose, or by using bioagents in combination with other agents or with enhancers, including low doses of chemical pesticides. Genetic variability of putative biopesticide organisms may be enormous — up to 2,900-fold differences in activity have been found among several geographical isolates of the NPV virus of gypsy moth (*Lymantria dispar*) (Shapiro, 1995). Genetic engineering of baculoviruses has been used to enhance their activity — time to kill the host has been reduced by 25–40% by expressing foreign proteins such as toxins (scorpion, mite, Bt toxins), insect hormones, and others (Moscardi, 1999).

The dose-mortality relationships of bioinsecticides have been recently reviewed and discussed by Evans (1999), who demonstrated the complicated nature of determining the required field dosage for obtaining adequate kill of the target pest. Differences in LD₅₀ between agents with identical LD₅₀ can be several thousand-fold, and vary also between life-stages of the pest. Increasing the dose of *Metarhizium anisopliae* for the control of the cabbage root fly larvae from 10⁷ to 10⁸ and to 10⁹ colony-forming units per plant increased the control (decreased the root damage) from 0 to 37 and to 70%, respectively (Vänninen *et al.*, 1999a), but still gave unsatisfactory field results (Vänninen *et al.*, 1999b).

Various substances can be included in the biopesticide formulation to enhance its activity. For example, the LD₅₀ for some formulations of the fungus *Beauveria bassiana* was reduced by 97% by the addition of coconut oil (Butt *et al.*, 1999). Baculovirus activity has been greatly enhanced by optical brighteners and other substances, including 'enhancin', a unique protein associated with the GV of rice armyworm (*Mythimna* (= *Pseudaletia*) *unipuncta*) from Hawaii (for a review see Moscardi, 1999). In Eastern European countries and in Russia *B. bassiana* has long been used together with a reduced dose of insecticides to control the Colorado beetle (*Leptinotarsa decemlineata*) (see Feng *et al.*, 1994); similar tactics can be used effectively with many other biopesticides, for example mycoherbicides (Pilgeram & Sands 1999).

It is not clear that the speed of kill of a pest is as important as it often is presented, and it even can be claimed that it would be more beneficial to target R&D at other features of the crop-pest interaction. Clearly, what is the most crucial parameter is the overall reduction in damage caused by the pest or pathogen and the duration of that effect — and these do not necessarily always correlate with the 'speed of kill'. Behavioural changes and sublethal effects in the target pest due to infections with pathogens have been documented to result in effective crop protection, even when the killing process has been slow. For example, Thomas *et al.* (1998) have shown that the impact of *Metarhizium* on the rice grasshopper *Hieroglyphus daganensis* was a significant reduction in feeding (thus, control effect of 20–50%) already on day 2, although no sign of infection or mortality was

evident. Similarly, spraying strawberry leaves in the laboratory with *Steinernema feltiae* and Bt *tenebrionis* resulted in only 7% and 15% leaf damage, respectively, in 24 hours, compared with 48% damage in water-treated controls by the leaf beetle *Galerucella sagittariae* (Hokkanen & Zeng, unpublished data). Thus, the cessation of feeding or causing of damage is important, and it may be beneficial for other natural enemies or for the horizontal transfer and secondary cycling of the pathogen that the mortality occurs more slowly. The critical importance of secondary cycling and horizontal transfer to the longer-term control by insect pathogens has been shown convincingly by Thomas (1999) and Thomas *et al.* (1999).

PERSISTENCE OF ACTIVITY

Most biopesticides are living organisms, and subject to population level processes once applied to the ecosystem. In addition, they often are placed into surroundings which are unusual to them — soil organisms (such as many fungi, nematodes) are applied to the foliage or on other surfaces, where they are exposed to physical extremes such as UV light, dessication, heat, etc. Thus, on one hand they tend to be rapidly inactivated by abiotic factors; on the other hand they may be rapidly attacked by antagonistic organisms. Once successfully established in the target ecosystem, however, they have a potential to multiply (secondary cycling) and to spread (horizontal transfer) in the target host population and to provide a long-term suppression of the target pest.

Major research effort has been targeted to improving the persistence of biocontrol agents after their use, and to obtaining an optimum coverage of the foliage or target system to be reached. Persistence of early Bt products used to be too short for effective control, but has now been optimised via formulation and in particular through genetic engineering techniques (microencapsulation) (Baum *et al.*, 1999). UV protectants (e.g. optical brighteners) are prolonging the activity of viral insecticides and innovative protective formulations that of fungal products (e.g. Moscardi, 1999; Wraight & Carruthers, 1999; Boyetchko *et al.*, 1999). The survival of entomopathogenic nematodes after application in the field is still very poor — the numbers tend to decline exponentially within hours and reach near-endemic levels in two weeks (see Glaser *et al.*, 1999).

Although adequate moisture is very important for the activity and persistence of many biopesticides, rain can quickly become detrimental if it washes off the organisms from the foliage. Inyang *et al.* (2000) have shown with different *Metarhizium anisopliae* formulations that simulated 1 h rain washes off conidia from oilseed rape leaves and reduces target pest mortality, but that conidial loss (compared with unexposed leaves) was affected by formulation: it was about 40% with one oil formulation, 61% with another oil, and 76% with conidia formulated in aqueous Tween. Persistence experiments with *M. anisopliae* showed, however, that when applied to the soil it remains infective and abundant enough for 3 years under Finnish conditions, in contrast to *B. bassiana* which disappeared within one year from all four tested soil types (Vänninen *et al.*, 2000).

SELECTIVITY OF ACTIVITY

A major difference to most chemical pesticides is the often very narrow specificity of biopesticides; some viruses are extremely host specific. This has often serious implications to the commercial attractiveness of the product; indeed, currently the most successful biopesticides are all relatively broad in their use and, thus, the potential market size is bigger. Narrow scope of activity is ecologically appealing, because biopesticides thus can easily be integrated to other management schemes, and unlike most chemical pesticides, they will not disrupt naturally occurring biological control of other pests. Further benefits in terms of field performance (*sensu lato*) of biopesticides include their safety to crop plants, application personnel, and lack of toxic residues on treated crops.

Current developments in this area include the selective broadening of the potential target spectra of several putative biopesticide organisms, often via genetic engineering. Thus, the rather broad-spectrum insect viruses (e.g. *Autographa californica* NPV) are engineered for enhanced virulence (Moscardi, 1999), and the same is attempted with a broad-spectrum microsporidian (*Vairimorpha necatrix*); most of the entomopathogenic nematodes and fungi under intensive product development have a relatively broad host range. Narrow-spectrum biopesticides will almost always necessarily remain as niche-market products, and will not be promoted by large industry.

Broadening the activity range of biopesticides, however, does have its limits; regulatory authorities and the public at large are currently very sensitive about non-target effects of biological pesticides, and if beneficial or rare and endangered insects — for example — will be put at risk through a broad-spectrum biopesticide, its use may never be registered and, therefore, there will be no market. Protocols for evaluating the non-target safety of biological controls are being established (e.g. EU-FAIR5-CT 3489 research project 'ERBIC' — see www.honeybee.helsinki.fi/mmsel/erbic.htm). Fortunately the research up till now indicates that biopesticides have only minimal non-target effects, and most agents have no measurable impact on important non-target organisms (Flexner *et al.*, 1986, Goettel *et al.*, 1990; Husberg & Hokkanen, 2000; Hokkanen & Zeng, unpublished data).

ECONOMIC PERFORMANCE

From the farmer's point of view the situation is clear; biopesticides must bring some tangible benefits over other crop protection systems in order to be adopted. In conventional farming they simply need to solve a problem for which there is no better, easier or cheaper alternative; in organic production the benefit may be added value of the product, or some other, less apparent aspect.

From the industry's point of view the situation is probably more complicated. Gelernter & Lomer (2000) present an interesting set of criteria for a successful biopesticide. It has to fulfil at least 3 or 4 of the following 5 requirements:

- technical efficacy
- practical efficacy
- commercial viability

- sustainability
- provision of public benefit.

Being good in just one or two of the criteria is not enough — no matter how well the product performs in the field, it will not be a commercial success unless it is easily adopted to the farming system, unless the company producing it accrues reasonable return on its investment, and unless it possesses still some additional beneficial features. In their analysis of several current and past bioinsecticidal products, the above arguments become quite clear — *Bt tenebrionis* has largely failed because it fulfils clearly only the first criterion, whereas *Bt israelensis* for the control of black flies (Simuliidae) appears to be successful (three criteria fulfilled) and *Bt kurstaki* for the tomato pest complex is clearly a successful biopesticide (4 criteria fulfilled).

So far, the biopesticide business has been disappointing for industry (e.g. Patel, 1991). With the exception of Bt, commercial microbe-based biopesticides are insignificant and probably all are still loss-making to the large companies involved in this area (Törmälä, 1995). Indeed, many medium-sized biopesticide companies have had to quit. On the other hand, a viable small-scale industry has sprouted especially in Europe (Butt *et al.*, 1999), while in the USA some medium-sized companies are still vigorously pursuing the biopesticide options. For example, Mycotech has recently registered several formulations of *B. bassiana* as the first mycoinsecticides in the USA for the control of insect pests in field crops, including the silverleaf whitefly (*Bemisia argentifolii*), and it is now considered that the '...advances in mycoinsecticide technologies ... have brought several species of entomopathogenic fungi to the verge of commercial success. ... The future looks extremely promising....' (Wraight & Carruthers, 1999 — p. 258).

Similar optimism prevails in reviews of other types of biopesticides — just as it did 10, 20, or 30 years ago — but this time, maybe with a slightly more sound scientific backing.

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Biological control in Integrated Crop Management – success factors

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Interpreting mycoinsecticide field performance: an uneasy relationship with chemical pesticide paradigms

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ABSTRACT

The term 'biopesticide' is most useful when applied strictly to living organisms that (a) are specific as individual products, and thus confer some environmental advantage (unlike many but not all chemicals), and (b) have a limited period of activity, and are therefore usually used with normal pesticide application techniques (unlike certain other biological control agents).

Although they have a reputation of being a 'soft option', biopesticides can work more effectively than their chemical equivalents in the long term; however, in order to be used successfully, a thorough understanding of how they interact with the biological target is required during product development.

Biopesticide products may arise out of the discovery of new agents or key technical fixes, such as the use of oil-based formulations to overcome the need for high humidity. However, in most cases there are key technical and practical hurdles (notably during the 'laboratory to field' process) which do have parallels with chemical product development. A good example occurs when the product must be sprayed, where the choice and adaptation of machinery follows basic rules: including the introduction of a minimal amount of novelty for the spray operator. Being particulate, a rigorous approach must be taken to the numerical aspects of mycoinsecticide-dose transfer, including: the concentration of particles in suspension, how particles are distributed in droplet size spectra, the mechanism of dose pick-up by the target and, often most crucially, the quality of the products themselves. These aspects are discussed using the development of two formulations containing two different *Metarhizium anisopliae* sub-species as case studies.

INTRODUCTION

The increasing concerns about chemical pesticides have heightened interest in alternatives such as biopesticides and pest-resistant, genetically modified (GM) crops. Recent public disquiet with the latter is as much related to ethical and political issues as to genuine scientific concerns that must be resolved within a broad ecological framework (Crawley, 1999). Biopesticides have been promoted for a longer time and, like other biological controls, carry certain risks (Thomas & Willis, 1998). Unlike GM crops, several biopesticides have a long-standing, relatively problem-free 'track record'; examples include: *Bacillus thuringiensis* (var. *kurstakii* — discovered in 1902), *Beauveria bassiana* (discovered in 1835) and *Metarhizium anisopliae* (discovered in 1878).

It is important to define here what we mean by the term 'biopesticide'. True biopesticides contain living organisms or propagules and have a limited persistence in the environment. They are therefore distinct from 'biorationals' such as non-living formulations based on the *B. thuringiensis* toxin and microbes acting as classical biological control agents (which may provide ideal control solutions: for example, in the management of invasive pest species). The latter are usually found in the pest species' original environment; after a period of safety screening, they can be introduced just once into the outbreak area, and are spread by secondary cycling or horizontal transmission. Copping (1998) provides an excellent overview of some 170 biology-based technical solutions including 'biorationals', pheromones, insect predators and genes for GM crops. Only a minority of these solutions constitute true biopesticides by our definition, and only 6 fungi are listed that have been commercialised as mycoinsecticides.

The market for mycoinsecticides is presently extremely small. Together with mycofungicides and mycoherbicides, fungal agents constitute only a minute proportion (some 3%) of the small world-wide biopesticide market (Georgis, 1997), which in turn remains limited to <1% of the total pesticide market (Lisansky, 1997). Lack of biopesticide promotion by authorities and the agrochemical industry has been attributed to: narrow target spectra, poor performance relative to cost and inconsistent product quality in comparison with chemicals. We believe that the two latter 'constraints' are misconceptions, given the appropriate scientific rigour and resources, and that if a genuinely integrated approach is taken to crop pest management (i.e. IPM/ICM), then a narrow target spectrum should be an advantage rather than a constraint. We shall attempt to illustrate these ideas with some experiences in the technical development of the fungus *Metarhizium anisopliae* in the form of ultra-low volume (ULV) sprays for locust and grasshopper control, and more recently as hydraulic sprays for a number of other insect pests. Thus, although this series of symposia is about relating laboratory to glasshouse to field performance, we see the important challenge to be the translation of success in a very specialised (ULV) application technique to mycoinsecticide delivery systems appropriate for the much larger field crops market.

LOCUST AND GRASSHOPPER CONTROL USING *M. ANISOPLIAE*

Amongst the mycoinsecticides, we have been especially interested in the use of various isolates of the Mitosporic (Deuteromycete) fungus *Metarhizium anisopliae* as insect control agents. With generous donor funding in the 1990s, the LUBILOSA Programme^{*} was able to develop an acridid pest control product for environmentally sensitive areas (FAO, 1998). 'Green Muscle'[®] is based on one particular isolate of *M. anisopliae* var. *acridum*: IMI 330189, which has been tested against a range of locusts and grasshoppers (Lomer *et al.*, 1999) and is now registered in South Africa. A similar isolate of *M. anisopliae* var. *acridum* has been field tested successfully in Australia (Milner, 1997) and will be marketed as 'Green Guard'. Both of these isolates have previously been called *M. flavoviride*, but they have been re-classified as a genetically homologous clade of *M. anisopliae* that is specific to grasshoppers and locusts (Driver *et al.*, 2000).

^{*} Lutte Biologique contre les Locustes et les SAuteriaux: a collaborative, multi-disciplinary research and development programme funded by the Governments of Canada (CIDA), the Netherlands (DGIS), Switzerland (SDC) and the UK (DfID)

In almost all cases, the delivery system for these fungal agents relies on the use of oil formulations. The enhanced mycoinsecticidal efficacy of oils in comparison with aqueous suspensions was confirmed with *M. anisopliae* var. *acridum*, in laboratory studies on desert locusts (Bateman *et al.*, 1993). Field efficacy has been demonstrated repeatedly at low daytime humidities; thus, oils effectively overcome the formerly supposed need with mycoinsecticides for high ambient humidity. Oil-based formulations have the added advantage of compatibility with standard ultra-low volume (ULV) spraying equipment already in use against locusts and grasshoppers.

In a recent series of operational trials against Senegalese grasshoppers (*Oedaleus senegalensis*) it was shown that although the organophosphorus chemical fenitrothion achieved an impressive 'knock down', hopper populations recovered two weeks after application. On the other hand, a more profound population reduction was achieved in comparable plots sprayed at ULV rates with *M. anisopliae* var. *acridum* conidia (Figure 1; Langewald *et al.*, 1999). This aerial application required rigorous quality standards in order to distribute 100 g of conidia at 0.5 litres/ha.

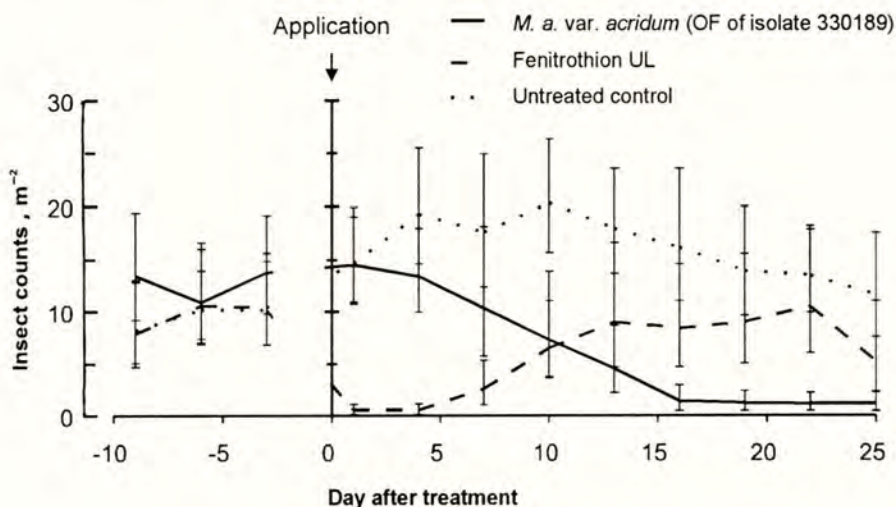


Figure 1. Results of an 800 ha aerial trial against *Oedaleus senegalensis* in East Niger (from Langewald *et al.*, 1999). Insect population counts were taken at 3-day intervals, before and after application of ULV formulations of *M. anisopliae* var. *acridum* and fenitrothion.

By monitoring efficacy over weeks (rather than days), this mycoinsecticide is substantially more efficacious than its main chemical rival. However, this overall effect is the product of a number of interacting factors. Field observations indicate that spores may persist in the field for several days, depending on conditions (Thomas *et al.*, 1997b). After the death of the insects, further conidia may be released as cadavers are broken down and, under suitable conditions, secondary cycling of inoculum can also reduce acridid populations

(Thomas *et al.*, 1995). Residues of the fungus are more persistent but have minimal impact on non-target organisms, which may also enhance field efficacy (Peveling *et al.*, 1999).

A reduction in feeding has been observed (Thomas *et al.*, 1997a) in a field-cage trial on *Zonocerus variegatus*, with a significant reduction in feeding 2–3 days post inoculation. These authors suggested that the reduction in feeding could be a trade-off between defence and reduced feeding, which contributes to crop protection. Further studies showed that one of the most important defence mechanisms in the field was insect thermoregulation. Blanford *et al.* (1998) found that infected *O. senegalensis* raised their body temperatures by approximately 3°C in comparison with uninfected individuals (see Figure 2), which could prolong the disease incubation period. Nevertheless, most acridids appear to be unable to completely suppress *Metarhizium* infections by behavioural fever and a few species, for example, *Zonocerus variegatus*, appear to be unable to control body temperature (Blanford *et al.*, 2000). Thus, the speed of kill is likely to be affected by the target species and by environmental conditions. This will increase the complexity of predicting the outcome of control campaigns using biological control methods.

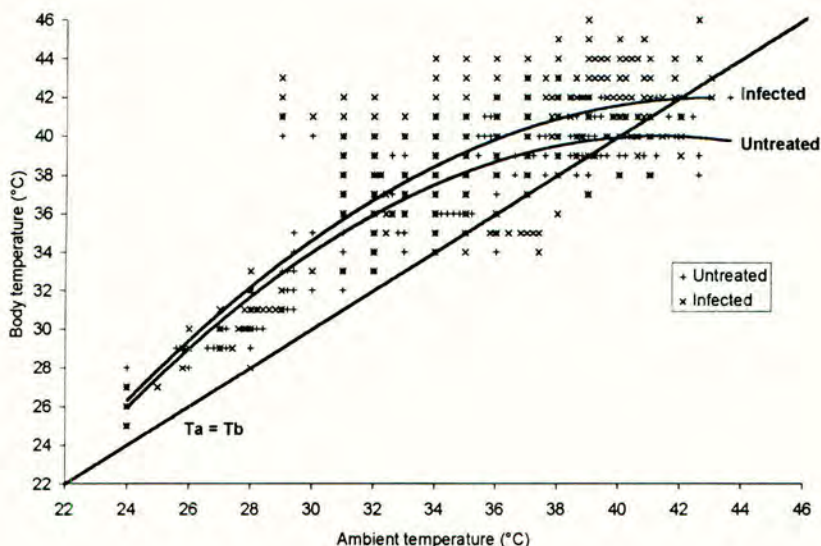


Figure 2. Effect of infection by *M. anisopliae* var. *acridum* on the thermoregulation of *Oedaleus senegalensis* (from Blanford *et al.*, 1998). The diagonal line indicates where insect body (T_b) and ambient (T_a) temperatures would be the same (i.e. if no thermoregulation had taken place).

PREPARATION OF OIL-BASED MYCOINSECTICIDE FORMULATIONS

Key practical considerations (Quality Assurance of product)

As with chemicals, a consistent high product quality is an essential feature of any reliable biopesticide (e.g. Jenkins *et al.*, 1998). From a safety point of view, biological purity of the product is of utmost importance during the mass-production phase. It is at this time that contaminants can out-compete the fungus and utilise the substrate (this problem is especially acute with some *Metarhizium* isolates that are relatively slow growing). The other key biological issue is the maintenance of spore rigour, which is dependent on temperature during the growth stage, drying time and the final moisture content of the fungus. High initial viability is important, and all further stages in the delivery system (storage, formulation and application) can be seen as potential 'weak links' that may affect the final vigour of the biological agent.

The moisture content of a fungal preparation, is important for the storage of the product. It is well documented (Moore *et al.*, 1995) that the moisture content of fungal spores dramatically affects storage time. Spores stored at higher moisture contents have reduced viability, and eventually die earlier than those fungal spores stored at around 5% moisture content. Too low a moisture content also damages the spores, so a suitable balance has to be achieved.

Application techniques must not damage the product, and this must be checked in the laboratory (e.g. Griffiths & Bateman, 1997) or in preparatory field trials (e.g. Bateman *et al.*, 1998) to assess the potential field performance of the biological pesticides. At ULV application rates, the quality of both the formulation and emitted spray droplet spectrum are crucial to maximise the distribution of very small amounts of biopesticide. An analyser of particle size is used to check that no large (>100 µm) material is present that might block filters or restrictors. Ideally, the preparation should consist of single conidia; further specifications for particle size (>80% by volume must be <10 µm) have been included to minimise settling in suspension formulations. Machinery has been developed that is capable of separating aerial conidia, from solid substrates, to produce preparations that conform to these specifications (Bateman *et al.*, submitted).

Materials and Methods

Droplet and particle size spectra were measured with a Malvern¹ 2600 particle size analyser. The instrument was fitted with lenses of 63, 300 or 600 mm, depending on the purpose of the experiment. Each reading comprised 1,000 scans (equivalent to sub-samples). Measurements were repeated at least once on a separate occasion to check for consistency and are presented here as means. Numbers were exported electronically into a database in the form of cumulative volume distributions over 32 size classes.

For measurements of particle size the instrument was fitted with a 63 mm lens, using model independent analysis and a PS1 sample cell that contained a small magnetic stirrer. Each reading consisted of a background measurement with either Shellsol T (Alcohols

¹ Malvern Instruments Ltd., Spring Lane South, Malvern, Worcs., WR14 1AT, UK

Ltd.) or distilled water, followed by the gradual introduction of concentrated suspensions (conidia or emulsions using a pipette. A reading was taken when the obscuration of the laser was optimal in the 'illustrate live' command.

Rotary nozzles were positioned approximately 250 mm in front of the laser beam for analysis of droplet size, and the instrument fitted with a 300 mm lens for the 'Ulva+' and a 600 mm lens for the 'X10'. A 300 mm axial fan situated at the rear of the apparatus drew spray away from the sampling area (in order to minimise operator exposure to spray and prevent small droplets re-circulating in the beam).

Development of ULV formulations

Figure 3 shows the particle size spectrum of a standard *M. anisopliae* var. *acridum* spore preparation, overlaid onto the droplet size spectra of two standard ULV atomisers spraying a formulation consisting of 100 g conidia/litre suspended in 50% Ondina EL oil (Shell Oils Ltd.) mixed with Shellsol T. Spraying ULV formulations for locust control usually requires a droplet size of approximately 40–120 μm diameter and the Ulva+ sprayer achieves >80% of the spray volume in this range, over a fairly wide range of rotational speeds (Bateman & Alves, 2000). The Micron 'Ulvamast' operates at much higher flow rates (and typically can treat >10 $\times$ the amount of land per day) but this is achieved at the expense of a slightly wider droplet spectrum. Good-quality formulations consist of practically all single spores and, at an operating concentration of 5×10^{12} conidia/litre, using 1 litre/ha, most droplets contain in the region of 500–10,000 conidia. Only a very few droplets need to be encountered by target acridids in order to receive a dose that is lethal within 2–3 weeks.

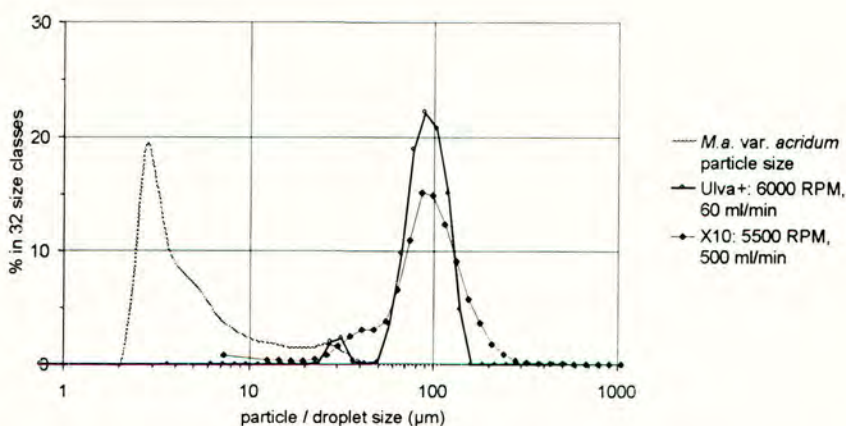


Figure 3. Particle size spectrum of a *M. anisopliae* var. *acridum* spore preparation, and the droplet size spectra of the oil-based formulation, produced by two ULV atomisers: the Micron Ulva+ and X10 (which is fitted to the 'Ulvamast').

The aerial trial described above used a newly developed oil miscible flowable concentrate (OF) of *M. anisopliae* var. *acridum*; this is a liquid containing 500 g conidia/litre and diluted with either diesel fuel oil or kerosene (paraffin), using simple mixing ratios. Although expensive, the OF formulation is much easier to handle than dry spores and is, therefore, quicker to prepare; it also avoids the risk of dust inhalation and makes more efficient use of conidia, thus reducing waste.

Emulsifiable formulations

In the context of world-wide pesticide use, ULV spraying represents a very specialised delivery system, and in order for mycopesticides to develop further, the use of oil-based formulations with hydraulic and other nozzles will be necessary. Growers of field crops are unlikely to accept novel agents, if this entails major modification or replacement of their application machinery (Chapple & Bateman, 1997). A readily available method for applying mycoinsecticides with hydraulic sprayers, at higher volume application rates (VAR) is to mix spores in proprietary emulsified spray oil adjuvants. The mixture is then added to an appropriate amount of water to form a 'spores-in oil-in water' tank mixture. Alves (1999) investigated a range of adjuvants and found that damage to *Metarhizium* conidia was least likely with products containing non-ionic surfactants.

Mycoinsecticides will develop from their presently specialised markets, and become acceptable to growers of field crops, only if they are made compatible with commonly available application equipment. In practice this usually means the use of sprayers based on hydraulic atomisers; therefore, in order to obtain the benefits of oil, emulsifiable suspensions of fungal agents appear to be required. Such formulations are analogous to the widely used suspension concentrate (SC) formulations sold by chemical companies. The principal technical issues in developing such formulations include: achievement of stable suspensions with small sized solid particles (as with SCs) and oil globules (as in ECs), maintaining contact between the oil and spores and biological viability of the fungus. With a chemical agent, small particle size can be achieved by milling, but a less harsh technique must be used with fungi — at the spore separation stage (Bateman *et al.*, submitted).

Figure 4 shows the distribution of particle size of a dry spore preparation of a commercial isolate of *Metarhizium anisopliae* var. *anisopliae*, measured using a 'Malvern' particle size analyser (PSA) fitted with a liquid sample cell. The bimodal size distribution is derived from the cylindrical shape of conidia of the isolate used in this example. The figure also shows how the PSA technique can distinguish between the 'globule size spectra' of different emulsifiable formulations (each having been mixed with distilled water). A commercial, emulsifiable mycoinsecticide formulation contained a high proportion of large, suspended matter in comparison with an experimental formulation the containing the *M. a. anisopliae* conidia shown above.

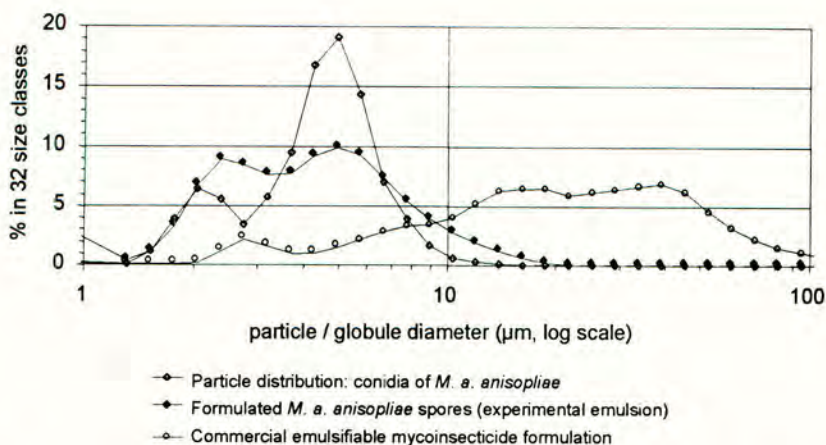


Figure 4. Particle and globule size spectra of a spore suspension of *M. anisopliae* var. *anisopliae* conidia as a dry powder, and an experimental emulsion (from the same preparation). This is compared with a commercial, emulsifiable mycoinsecticide formulation.

In order to maintain a protective layer of oil around each spore (as in ULV formulations) the solid and oily components of emulsifiable suspensions of mycoinsecticides should not separate out. Unfortunately, separation may occur with presently available commercial mycopesticides, but by using an appropriate choice of surfactants this can be prevented (as with the experimental *M. a. anisopliae* formulation illustrated). The stability of an emulsion is usually linked to the globule size, and are assessed with standard techniques such as those described in the Collaborative International Pesticides Analytical Council (CIPAC) handbook (1994). However, such tests are designed for chemical pesticides, and problems may occur in trying to relate the standardised tests to biological pesticides. For example, the method to test emulsion characteristic of emulsifiable concentrates (MT 36 in the CIPAC handbook F) is quoted as 'not always suitable for emulsifiable concentrates containing solid active ingredients'. Alternative methods are under consideration but to date have not been released. When these globules must incorporate fungal agents there is obviously a lower size limit, set by the size of spores themselves. This makes it difficult to compare these with chemical pesticides. Although these methods can be used to test biological pesticides, it may never be possible to apply the physical stability standards of chemicals to emulsifiable mycoinsecticide formulations.

The use of formulating oils enhances infectivity, reduces the need for high humidity and may provide some additional protection against factors such as ultra violet radiation. Since it would be impractical and uneconomic to use oils in conventional medium- to high-volume application equipment, the use of oil emulsions is seen as a key technical solution to the development of mycoinsecticides in field crops. However, trials with *Beauveria bassiana* products in N. America have produced indifferent results (Wraight &

Carruthers, 1999). Furthermore, oil alone was more efficacious than oil emulsion with a mycofungicidal preparation of *Verticillium lecanii* (Verhaar *et al.*, 1999), which might apply similarly to the insecticidal isolates of this fungus ('Vertalec' and 'Mycotal'). As we have shown, interpretation of mycoinsecticide field results is even more complicated than with chemical insecticides, and further research will be needed before spore emulsions can be adopted or dismissed. Delivery systems must not be seen as solutions to problems with microbial agents; however, mycopesticides will be ineffective if an inadequate dose of live propagules is transferred to the target.

DISCUSSION AND CONCLUSIONS

There are great dangers when applying a 'chemical paradigm' to biological products which, unlike chemical pesticides, are based on living organisms. The obvious disadvantage of biological pesticide products is the crucial need to maintain their viability from the production unit to the biological target. However, there is also a danger that biopesticide performance may be seriously undervalued in the field — especially in the long term.

Useful parallels can be drawn between chemical and mycological insecticides in delivery system technology and in commercial development. Biopesticides applications will probably fail without good-quality control, rigorous calibration etc., ensuring that an adequate dose of live propagules is transferred from the fermenter to the target. A similar statement can be made about chemical products; however, although they possess considerable expertise, biopesticide production by large pesticide companies has been disappointingly little to date.

There are numerous examples in the literature on the potential of various microbial agents for ecologically sound pest management. However, there are practically no examples of this rather academic approach resulting in effective biological control operations in the field. Little progress will be made unless viable, practicable products are made available to growers. It has been pointed out by a number of research groups and authors (including Boyetchko, 1999), that the best (and perhaps the only sustainable) model of biopesticide development appears to be one where small- to medium-sized commercial producers are linked with, and back-stopped by, public institutions. The development of *M. anisopliae* var. *acridum* by the LUBILOSA Programme and the links between the former Glasshouse Crops Research Institute (now HRI) and producers of the mycoinsecticide *Verticillium lecanii* provide good examples.

Biopesticide action, therefore, contrasts with that of chemicals, but expertise common to both types of pesticide is required for registration, distribution and marketing of products. There are also common delivery systems (formulation, packaging and application techniques), but unfortunately with biopesticides this subject is too often a 'no man's land' when it comes to support and implementation (Bateman, 1998).

Commercial pressures have encouraged the excessive promotion of single technology ('magic bullet') concepts, that have not delivered all that was promised. It is the use of techniques in combination with one another that offers the greatest potential for farmers to reduce costs and to impact on non-target organisms. Substantial improvements to

conventional practice can be made using a rational pesticide use (RPU) approach; this is the sub-set of IPM, in which the adverse effects of pesticide use can be mitigated by improvements in the selectivity of the products themselves and in the precision of their application in both space and time.

Mycopesticides can be seen as a tool for developing a more rational pesticide use strategy, and (like chemicals) new products have followed key technological developments. Their success or otherwise should be measured only against a clearly defined role for such products. Scientists having a chemical background need to understand the implications of working with living organisms; perhaps more importantly, biological control/IPM practitioners must understand that in the real world mycoinsecticide products must be available and practicable if they are to have any future.

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Controlling plant pathogens and improving plant growth and productivity with biologicals

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ABSTRACT

Recent advances with biocontrol fungi in the genus *Trichoderma* reveal new mechanisms by which they control plant-pathogenic fungi and improve plant growth and productivity. Within the past 2–3 years, the number of known general mechanisms has increased from three to eight. This knowledge, together with data from thousands of commercial and university field trials, indicates that the benefits from these fungi are much larger than heretofore expected. However, these mechanisms are invariably complex, poorly understood and no doubt multigenic. Relatively rapid increases in the level of use of products based on these fungi, and their reliability, are expected. These increases are based on greater understandings of mechanisms and limitations, as well as registrations in an increasing number of countries and improved, mechanism-based marketing efforts. However, the greatest advances in commercial use and reliability await holistic understandings of the function and regulation of the genes of these fungi from genomics-based approaches.

INTRODUCTION

Biological control of plant pathogens and plant-growth-promoting biological agents have been described frequently but still are not widely used in production agriculture. In terms of efficacy, there are several important parameters that must be met, including the following:

- The strains/products employed must perform reliably.
- The limitations and expectations of the biological agent must be realistic and conveyed accurately to the end user.
- The general mechanisms of action and the range of pathogens controlled/advantages conferred must be understood.

Of course, in addition to the factors noted above, other aspects also must be in place if wide-scale use is expected, including quality-production techniques and facilities, robust and reliable quality control and standard operating procedures, and a marketing system/staff that understands and conveys both the advantages and disadvantages of the particular product.

Perhaps the best characterized and documented biocontrol/plant-growth-promotive agent is *Trichoderma harzianum* strain T-22 (a.k.a. 1295-22, KRL-AG2, ATCC 20847). This strain was produced using protoplast fusion (Harman *et al.*, 1989; Stasz *et al.*, 1988). Products based on this strain are registered and sold commercially in the USA, and elsewhere, and a great deal of the knowledge of this organism has been derived from the thousands of commercial trials. Products based on this strain are listed for use with organic growers, have

0-hr re-entry periods and are exempt from residue tolerance (Harman, 2000). Retail sales of products based on this organism totalled around \$4 million in the past twelve months (BioWorks data, Geneva, NY; see also <http://www.bioworksbiocontrol.com/>).

Much of this document will describe the abilities and properties of this and other strains of *Trichoderma*, as well as limitations. Most of the features noted are well documented in terms of actual field performance. In addition, this paper will also feature general mechanisms of biocontrol by *Trichoderma* spp. and indicate why mechanistic information is critical to expectations of field performance. The reader is also referred to Harman (2000), which further documents these points.

Trichoderma spp. are extremely common, no doubt constituting a lynch pin component of most soil and other ecological communities. These cosmopolitan fungi are present in essentially all soils. Frequently found at levels of 10^2 to 10^4 colony-forming units per g (Lo *et al.*, 1996; Klein & Eveleigh, 1998), they often are the most prevalent culturable fungi from soils. Nonetheless, some strains are much 'better' than wild strains and have been commercialized (Harman & Björkman, 1998; Harman, 2000).

Rhizosphere competence

Some strains of *Trichoderma* are strongly rhizosphere competent. Rhizosphere competence is defined as the ability of a microorganism to grow and function in the developing rhizosphere (Ahmad & Baker, 1987). Strains differ remarkably in this ability; many strains have the capacity to colonize roots locally following chance encounters, but the very best will spread over the surface (Harman, 2000) or into the cortex (Yedidia *et al.*, 1999) of developing roots, covering the entire root surface in a protective but invisible mantle of hyphae (Harman, 2000). A high level of rhizosphere competence permits a number of uses of considerable agricultural relevance (see below).

MECHANISMS OF BIOCONTROL

Until very recently, only three mechanisms of biocontrol by these fungi were generally accepted (mycoparasitism; antibiosis; competition for nutrients or space), although promotion of plant growth was generally recognized as an additional attribute (Chet, 1987). Recently, a number of new mechanisms have been identified (from Harman, 2000):

- tolerance to stress through enhanced root and plant development;
- solubilization and sequestration of inorganic nutrients;
- induced resistance;
- inactivation of the pathogen's enzymes.

As noted above, a number of mechanisms of biocontrol have been documented within different *Trichoderma* strains. No single strain is known to possess all of these mechanisms and the genetic and biochemical bases of the activity of any strain are poorly understood. Where partial genetic bases of mechanisms are known, the processes involved appear highly multigenic. An intriguing phenomenon noted more than once, and documented below and in more detail in Harman (2000), is that genetic manipulation to reduce or eliminate one mechanism of biocontrol may result in the modified strain expressing a separate and unrelated

mechanism. Again, this complexity, probably, can be understood most efficiently with holistic genomics approaches, while reductionist biochemical and genetic approaches have thus far not proven satisfactory.

Understanding this wide range of potential mechanisms should strongly affect any consideration of field efficacy. For example, Deacon (1994) stated that biological control agents 'achieve only transitory localised dominance of the rhizosphere, and in only some soils and seasons...'. Similarly, Mathre *et al.* (1999) indicate that nearly all commercialized microorganisms rely upon application of the antagonist 'directly and precisely to the infection court when and where needed.' Neither is true for a strongly rhizosphere competent organism, which can reliably colonize and confer advantages to crops for several months after application (Harman, 2000). Similarly, Mathre *et al.* (1999) state that 'One rather daunting principle that applies across all biological methods for disease and pest control with introduced agents ... is that, almost invariably, a different agent ... is needed for each disease or pest.' Again, this is not true for organisms with a wide range of mechanisms/gene products involved in biocontrol. Most *Trichoderma* spp. can control a wide range of plant pathogenic fungi. A brief summary of some mechanisms follows.

Mycoparasitism

Mycoparasitism is a complex process that includes tropic growth of the *Trichoderma* strain towards the target fungus, lectin-mediated attachment of the two fungi and induced secretion of both cell-wall-degrading, and other, enzymes (Chet *et al.*, 1998). Antibiotics such as peptaibols probably also are involved (Schirmböck *et al.*, 1994). The enzymes most frequently considered to be involved are those that degrade cell walls, e.g. chitinases and β -1,3 glucanases. We now know that for each of these two functional groups there are multiple classes of enzymes and within each class there are distinctly different enzymes. In 1998, Lorito listed ten separate chitinolytic enzymes alone (Lorito, 1998). Similar levels of diversity exist with β -1,3 glucanases (Benitez *et al.*, 1998). In addition, β -1,6 glucanases (Lora *et al.*, 1995) and proteases (Geremia *et al.*, 1993) are probably also involved. For mycoparasitism of pythiaceae fungi, β -1,4 glucanases may also be important (Thrane *et al.*, 1997). Therefore, a single step in one mechanism - the mycoparasitic process of *T. harzianum* - may involve >20 (!) separate genes and gene products under complex regulatory control (Zeilinger *et al.*, 1999). Further, most of these gene products are synergistic with one another (see Lorito, 1998, for a summary). Given this entire arsenal of synergistic gene products that are part of only one mechanism by which *Trichoderma* spp. attack and gain nutrition from other fungi, it is very clear that a large number of genes are involved in biocontrol. Given this wide range of gene products, it is not surprising that *Trichoderma* spp. have abilities to control a wide range of plant-pathogenic fungi.

Antibiosis

A large number of substances toxic to other microbes have been obtained from *Trichoderma* spp.; 43 different antibiotics were listed in a recent review (Sivasithamparam & Ghisalberti, 1998). Of these, alkyl pyrones, diketopiperazines, isonitriles, peptaibols, polyketides, sesquiterpenes and steroids have often been associated with biocontrol of some species of *Trichoderma*. Like most characteristics of these fungi, the production of specific compounds may be limited to specific strains or species. For example, gliotoxin is produced by many strains of *T. virens* (Howell, 1998) but does not appear to be produced by *T. harzianum*.

Again, this wide range of fungal metabolites adds to the tools that these fungi have evolved in order to compete with other microbes, so we can use them to provide wide-ranging and broad-spectrum biocontrol agents.

Induction of resistance

Some strains of *Trichoderma* can directly induce resistance in plants. In an effort to investigate biocontrol mechanisms, Howell *et al.* (2000) produced UV mutants of *T. virens* deficient in their abilities to produce gliotoxin or to be mycoparasitic on other fungi. Surprisingly, some of the mutants had a greater ability to protect cotton seedlings against *Rhizoctonia solani* than the parental strains, because they induced production of antifungal terpenoids (such as gossypol) and related compounds in the cotton plants to which they were applied (Howell *et al.*, 2000).

These resistance-inducing strains, therefore, appeared to compensate for the loss of some biocontrol mechanisms by induction of others, again demonstrating the rich diversity of agriculturally useful genes within these fungi and their ability to adapt rapidly to genetic changes. The protein(s) that induces the resistance response in plants is now being identified (C R Howell, unpublished).

Similarly, Yedidia *et al.* (1999) examined the relationship between a strain of *T. harzianum* and cucumber seedlings in axenic culture. The strain invaded the cortex of the seedlings and induced an array of features indicative of induced resistance, including deposition of cell wall barriers and strengthening of cortical and epidermal cell walls. Peroxidase and chitinase activities were increased both in root and leaf tissues, which is indicative of systemic acquired resistance.

Inactivation of the pathogen's enzymes

Trichoderma spp. have abilities to control foliar pathogens as well as those in soil. Control of powdery mildews, probably, is primarily a function of induced resistance (Elad *et al.*, 1999). However, other mechanisms also exist. Conidia of *Botrytis cinerea* must produce enzymes (such as pectinases) that degrade cell walls, in order to infect leaves. *Trichoderma* spp. on leaves produce proteinases that inactivate the pathogen's enzymes and, therefore, prevent infection (Elad & Kapat, 1999).

Advantages of *Trichoderma* for biological control of plant pathogens

So far as we are aware, *Trichoderma* strains have been found that are capable of controlling every fungal disease for which control has been sought. Strain T-22 of *T. harzianum* is capable of controlling *B. cinerea*, *Cylindrocladium* spp., *Fusarium* spp., *Gaeumannomyces graminis* var. *tritici*, *Myrothecium* spp., *Pythium* spp., *R. solani*, downy mildews and powdery mildews (Harman, 2000). It does not, however, control *Phytophthora* spp. but *T. virens* controls this pathogen (Smith *et al.*, 1990) as well as most of the pathogens noted above. This wide-ranging ability of *Trichoderma* spp. to control pathogens is probably a consequence of the plethora of effective genes and gene products contained within these organisms.

ADVANTAGES TO PLANTS CONFERRED BY ENHANCED ROOT AND PLANT DEVELOPMENT

Trichoderma spp. have been known for several years to increase plant growth and development (Chang *et al.*, 1986). Several different lines of evidence suggest that this enhancement is not just via control of clinical and subclinical plant pathogens but is instead related to direct effects on the plant. These effects include the ability of specific strains to induce rooting in a manner analogous to rooting hormones (Harman, 2000) or to increase plant growth and development in axenic plant culture (Yedidia *et al.*, 1999).

In the field, such enhancement can be dramatic. Not only is the superficial root mass increased but also deep roots may be enhanced. In our trials, a seed treatment with *T. harzianum* resulted in mature plants (2 m tall) that had twice as many roots 60 to 100 cm below the soil line as plants not treated with *T. harzianum*. Similar plants in field trials had substantially increased drought tolerance (Harman, 2000).

Enhanced efficiency of nitrogen fertilizer use

Corn seeds treated with *T. harzianum* produced plants that gave maximum yield with about 40% less nitrogen fertilizer than plants without the symbiotic fungus (Harman, 2000). This difference is extremely important; the Environmental Protection Agency is required by law (<http://www.gove/mbasin/legis98.html>) to develop a plan to reduce the 'Dead Zone', a region of hypoxia in the Gulf of Mexico. Groups such as the American Farm Bureau are strongly opposed and concerned (<http://www.fb.com/2000annual/amnews/hypoxia.html>) since mandated reductions in nitrogen fertilizer (likely to be part of the plan) will probably reduce farm profitability and yield. Clearly, *T. harzianum* has the capability to provide a tolerable solution to this difficult dilemma.

Solubilization and sequestration of inorganic nutrients

In addition to nitrogen, other plant nutrients (such as metals and phosphorus) may be present in insoluble forms and, hence, are unavailable to plants. Metals, including Cu, Fe, Mn and Zn, frequently are present in oxidized forms, especially in alkaline soils, and P usually is complexed with Ca, Fe or with other species and also is insoluble. *T. harzianum*, at least, has the capabilities to dissolve the insoluble forms of these plant nutrients (Altomare *et al.*, 1999). Some of these, such as Mn, are essential to the plant's ability to resist disease; providing Mn to the plant to enable its native resistance mechanisms could be considered a potential biocontrol mechanism. Further, the chemical species produced by the fungus to accomplish these tasks are many. Every metal is solubilized by different chemicals and Fe, at least, is solubilized, by reduction from Fe^{+3} to Fe^{+2} and/or by at least multiple siderophores (Altomare *et al.*, 1999). Therefore, there must be numerous genes within the *Trichoderma* genome that encode either proteins directly involved in nutrient solubilization or enzymes involved in pathways for secondary metabolite synthesis.

REQUIREMENT FOR WIDER-SCALE USE

If *Trichoderma* spp. can accomplish all of the above, then why are they not used extensively to control plant diseases and to improve plant growth and productivity. Part of the answer is that most of these mechanisms have only just been discovered and uses based upon these have just begun.

However, other factors also are important. *Trichoderma* spp., like other biocontrol agents, have limitations inherent in their very nature. Understanding these limitations is as important as understanding the capabilities of the microbes. A listing of limitations of *T. harzianum* strain T-22 follows (from Harman, 2000). The products are management tools for growers; they are not magical, nor are they a 'silver bullet' that solves all problems.

- T-22 is strictly preventive. It cannot control existing diseases and so a good systemic fungicide must be used if diseases already exist.
- It is less effective against systemic diseases than more superficial ones. Therefore, it is more effective, for example, against *Fusarium* crown and root rot than against *Fusarium* wilts.
- In conditions of high, or very high, disease pressure, T-22 should be used as part of an integrated chemical-biological system. For example, for control of *B. cinerea* on strawberries in Florida, its best use is probably as a tank mix or as an alternating spray to reduce, but not eliminate, the chemical fungicide application.
- In other cases, maximum benefit to the crop requires use of both biological and chemical agents. For example, the combination of chemical seed treatment for maximum seed and seedling protection together with the long-term root protection and enhancement by T-22 is highly effective.
- While T-22 is extremely persistent on root surfaces, it does not persist at biologically significant levels in the absence of roots. Perhaps the only exception to this generalization is in highly organic soils like those in which onions are grown in upstate New York.
- T-22 does not always, or perhaps not even in the majority of situations, give obvious visual enhancement of plant growth or yield. T-22 provides tolerance to a variety of biological and edaphic stresses. If no stresses occur and plants are always growing at near-optimal conditions, T-22 can provide little visual, or yield, improvement.

Need for further mechanistic studies

Trichoderma spp. possess an almost bewildering array of mechanisms that influence microbe-microbe, plant-microbe, and microbe-microbe-plant interactions. This diversity of metabolites must be reflected in a similar diversity of functional genes. In fact, there must be hundreds of separate genes involved in biocontrol and plant growth responses. Their control is no doubt complex and is only just beginning to be understood.

As indicated earlier, much of the biochemical and genetic bases of the various mechanisms of biocontrol are poorly understood. No doubt, once understanding is gained, we will be much more capable of using these properties of agricultural and other commercial purposes.

For the first time, it is now possible to fully understand and manipulate complex genetic characteristics through functional genomic approaches. Such approaches permit examination of entire organisms on a holistic basis rather than using reductionist single metabolite or gene approaches that, thus far, have not yielded a comprehensive understanding of the function of any but the very simplest biocontrol agents. Once holistic genomics based approaches to complex biocontrol agents are complete, biocontrol will be able to be used much more effectively. Once our biocontrol agents are fully understood, we will be able to predict their performance much more accurately, genetically manipulate the agents to cause them to function as we wish, and use their genes and metabolites in a variety of agricultural and other commercial settings.

Consequently, an International *Trichoderma* Consortium is being organized. Its goals are:

1. To identify all of the genes and their functions within *Trichoderma* spp.
2. To understand how *Trichoderma* spp. function in nature and *in vitro*, via knowledge of their genes and gene expression.
3. To use the genes, gene products, and strains of *Trichoderma* for improvements in agriculture and other industries.

These goals can be achieved largely at a cost of about US \$7–10 million within a relatively short time frame. Once they are accomplished, field performance of biocontrol agents can be made much more reliable and biocontrol can become an important component of agriculture. In the meantime, much can be accomplished with existing agents, and we can expect them slowly to become adopted within horticulture and agronomy. Even our relatively poor understanding of these microbes will permit us to use them effectively, pending much greater improvements of performance possible once fully genomics-based understandings of our biocontrol agents is accomplished.

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Implementing biological control technology into the management of alien invasive weeds: South African experiences and challenges

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ABSTRACT

Since its inauguration in 1913, biological control of alien invasive plants has had an excellent track record in South Africa, but has rarely solved alien plant problems in isolation of other methods. However, biological control agents, notably insects, mites and pathogens, can augment the efficacy of conventional control methods if they are implemented and integrated appropriately. South Africa's 'Working for Water' (WFW) Programme, a national initiative geared towards the mechanical and chemical removal of alien plants from catchments and other water resources, has recognized the importance of biocontrol in sustaining progress over the long-term. Consequently, a National Biological Control Implementation Strategy (NBCIS) was formulated in 2000 and will be focused on (i) mass production and releases of new and poorly established biocontrol agents, (ii) collection and redistribution of well-established and effective agents, (iii) monitoring of the agents' establishment and assessments of their impact, and (iv) national co-ordination of the strategy. Challenges facing the NBCIS include (i) training and technology transfer to resolve practical problems, (ii) appropriate integration of control methods, (iii) conservation of biocontrol agents, and (iv) initiating preemptive biocontrol programmes. The potential savings to the WFW Programme, should biocontrol be implemented to its full potential, has been estimated at 59% of the total clearing costs (i.e. around £480 million), while the agricultural and environmental benefits are considerable.

INTRODUCTION

Biological control of weeds has been practised in South Africa for some 87 years and has involved 47 alien plant species that have invaded agricultural systems, commercial forests, water resources and conservation areas (Olckers, 1999; Table 1). The biological control agents implicated comprise herbivorous insects, mites and pathogens. Most programmes (72%) have focused on agricultural systems, where biocontrol has been confined largely to weeds of pastures and rangelands, with only one programme involving a crop weed (Table 1). However, since 1997, the emphasis has shifted from agricultural weeds to species that impact on water resources. South Africa is regarded as a 'water poor' country, with a large sector of the population not having access to adequate water supplies. Consequently, the protection of South Africa's water resources has become paramount, given the estimate that some 7% of the country's mean annual runoff (3.3 billion m³) is lost through transpiration by alien plant invaders (Versfeld *et al.*, 1998). Details on all biological control programmes that have been launched against weeds in South Africa are contained in several publications, comprising two review volumes (Hoffmann, 1991, Olckers & Hill, 1999).

Although weed biocontrol has had a high success rate (86% of evaluated programmes; Table 1) in South Africa, the degrees of success vary from instances of complete control (i.e. no other control measures are required to maintain the weed populations at acceptable densities) to substantial control (i.e. conventional control methods are still required, but at reduced rates). However, instances of complete biocontrol are exceptional in South Africa (31% of evaluated programmes) and biocontrol is regarded as a means of improving the efficacy of conventional control methods (Olckers *et al.*, 1998). Indeed, agents that cause even moderate levels of damage can contribute substantially to weed control, provided that they are integrated appropriately with other control methods. Consequently, it has become crucial that biocontrol be implemented appropriately and integrated in order to maximize the benefits of the agents.

These concepts are embodied in the strategy of the 'Working for Water' (WFW) Programme, a social upliftment scheme aimed at improving national water yields by the removal of woody plant invaders in catchments, riparian zones and wetlands. Inaugurated in 1996, this programme relies primarily on mechanical and herbicidal methods but has identified biocontrol as the only sustainable method to prevent the spread of alien invasive plants and the re-invasion of cleared areas in the long-term. Indeed, it was calculated that, if fully exploited, biocontrol has the potential to reduce the costs of the programme by 59% (£480 million) (Versfeld *et al.*, 1998). Consequently, the implementation of biocontrol technology has become crucial to the success of the programme and, thus, is embodied in the recently inaugurated National Biological Control Implementation Strategy (NBCIS).

In this paper, the elements and implications of the NBCIS are discussed, and some of the challenges that need to be addressed in order to facilitate the success of the venture in South Africa are highlighted.

BIOLOGICAL CONTROL OF SOUTH AFRICA'S WORST WEED INVADERS

The WFW Programme has compiled a list of South Africa's most invasive weeds, which includes some 66 species or groups of species (Versfeld *et al.*, 1998). Of the 25 highest-ranked species, based on total area invaded (Table 2), 15 have been exposed to biocontrol agents, while the remainder are mostly commercial or beneficial species that have not yet been targeted because of conflicts of interest. Although some 'beneficial' invaders are being considered for biocontrol (e.g. reducing the seeding capacity of *Pinus* spp.), most are unlikely to be targeted until legislation dictates that their negative impacts outweigh their benefits.

Of the 15 species already exposed to biocontrol, seven have been debilitated significantly by the agents which thus need to be included in future integrated control efforts (Table 2). Although, so far, the impact of biocontrol has proved negligible in two species, the remaining six species have been exposed to the agents only recently, further emphasizing the need for more efficient implementation to speed up the process. Although the NBCIS will be concerned primarily with the main weeds targeted by the WFW Programme, it will also be extended to include weeds of agriculture (e.g. the crop weed *Solanum elaeagnifolium*) and conservation areas (e.g. the weed *Leptospermum laevigatum*, which threatens the unique and endangered vegetation of the Cape Floral Kingdom).

Table 1. Implementation strategies for the 47 alien plant species subjected to biological control in South Africa.

Target weeds *	Resource affected **	No. of agents available	Control status ***	Strategy ****
ARACEAE				
<i>Pistia stratiotes</i>	Wat, Con	1 (insect)	Complete	Stages 2 & 3
ASTERACEAE				
<i>Ageratina adenophora</i>	For	2 (insect & pathogen)	Negligible	No action
<i>Ageratina riparia</i>	For	1 (pathogen)	Unknown	No action
<i>Chromolaena odorata</i>	Pas, Con, For, Wat	1 (insect)	Unknown	Stage 1
<i>Cirsium vulgare</i>	Pas	1 (insect)	Unknown	No action
<i>Silybum marianum</i>	Pas	1 (insect)	Unknown	No action
AZOLLACEAE				
<i>Azolla filiculoides</i> ⁿ	Wat, Con	1 (insect)	Complete	Stages 2 & 3
BIGNONIACEAE				
<i>Macfadyena unguis-cati</i> ⁿ	Con, For	1 (insect)	Unknown	Stage 1
CACTACEAE				
<i>Cereus jamacaru</i>	Pas	2 (insects)	Unknown	Stage 2
<i>Harrisia martinii</i>	Pas	2 (insects)	Complete	Stages 2 & 3
<i>Opuntia aurantiaca</i>	Pas, Con	2 (insects)	Substantial	Stages 2 & 3
<i>Opuntia dillenii</i>	Pas	1 (insect)	Unknown	No action
<i>Opuntia exaltata</i>	Pas	1 (insect)	Unknown	No action
<i>Opuntia ficus-indica</i>	Pas, Con	4 (insects)	Substantial	Stage 3
<i>Opuntia imbricata</i>	Pas	1 (insect)	Substantial	Stages 2 & 3
<i>Opuntia leptocaulis</i>	Pas	1 (insect)	Complete	No action
<i>Opuntia lindheimeri</i>	Pas	1 (insect)	Substantial	Stages 2 & 3
<i>Opuntia rosea</i>	Pas	1 (insect)	Substantial	Stages 2 & 3
<i>Opuntia salmiana</i>	Pas	1 (insect)	Substantial	Stages 2 & 3
<i>Opuntia spinulifera</i>	Pas	1 (insect)	Unknown	No action
<i>Opuntia stricta</i> ⁿ	Pas, Con	2 (insects)	Substantial	Stage 2
<i>Opuntia vulgaris</i>	Pas	3 (insects)	Complete	Stage 3
<i>Pereskia aculeata</i>	Con, For	1 (insect)	Unknown	Stage 1
CLUSIACEAE				
<i>Hypericum perforatum</i>	Pas	2 (insects)	Complete	Stage 3
CONVOLVULACEAE				
<i>Convolvulus arvensis</i>	Pas	1 (mite)	Unknown	No action

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Table 1 (continued).

Target weeds *	Resource affected **	No. of agents available	Control status ***	Strategy ****
FABACEAE				
<i>Acacia cyclops</i>	Pas, Con, Wat	1 (insect)	Substantial	Stage 2
<i>Acacia dealbata</i>	Pas, Con, Wat	1 (insect)	Unknown	Stage 2
<i>Acacia longifolia</i>	Pas, Con, Wat	2 (insects)	Substantial	Stage 2
<i>Acacia mearnsii</i>	Pas, Con, Wat	1 (insect)	Unknown	Stage 2
<i>Acacia melanoxylon</i>	Pas, Con, Wat	1 (insect)	Substantial	Stage 2
<i>Acacia pycnantha</i>	Pas, Con	1 (insect)	Substantial	Stage 2
<i>Acacia saligna</i>	Pas, Con, Wat	1 (pathogen)	Complete	Stage 3
<i>Caesalpinia decapetala</i> "	Pas, Con, For, Wat	1 (insect)	Unknown	Stage 1
<i>Leucaena leucocephala</i> "	Con	1 (insect)	Unknown	Stage 1
<i>Paraserianthes lophantha</i>	Pas, Con	1 (insect)	Substantial	Stage 2
<i>Prosopis</i> spp.	Pas, Wat	2 (insects)	Negligible	No action
<i>Sesbania punicea</i>	Con, Wat	3 (insects)	Complete	Stages 2 & 3
HALORAGACEAE				
<i>Myriophyllum aquaticum</i>	Wat	1 (insect)	Substantial	Stage 2
MYRTACEAE				
<i>Leptospermum laevigatum</i> "	Pas, Con	2 (insects)	Unknown	Stage 2
PONTADERIACEAE				
<i>Eichhornia crassipes</i> "	Wat, Con	6 (insects, mite & pathogen)	Substantial	Stages 2 & 3
PROTEACEAE				
<i>Hakea gibbosa</i>	Con, Wat	1 (insect)	Negligible	No action
<i>Hakea sericea</i>	Con, Wat	2 (insects)	Substantial	Stages 2 & 3
SALVINIACEAE				
<i>Salvinia molesta</i>	Wat, Con	1 (insect)	Complete	Stages 2 & 3
SOLANACEAE				
<i>Solanum elaeagnifolium</i>	Cro, Pas	2 (insects)	Substantial	Stage 2
<i>Solanum mauritianum</i> "	Pas, Con, For, Wat	1 (insect)	Unknown	Stage 1
<i>Solanum sisymbriifolium</i>	Pas, For	1 (insect)	Unknown	Stage 2
VERBENACEAE				
<i>Lantana camara</i> "	Pas, Con, For, Wat	6 (insects)	Negligible	Stages 1 & 2

* Programmes involving new agents " (i.e. released during and after 1996).

** Resources affected include croplands (Cro), pastures & rangelands (Pas), conservation areas (Con), forestry plantations (For) and water resources including catchments (Wat).

*** Complete = no other control methods needed, Substantial = other methods still needed, but at reduced rates; Negligible = other methods still needed at the same rates; Unknown = recent or unevaluated projects.

**** Stage 1 = mass-rearing and releases, Stage 2 = redistribution of agents, Stage 3 = monitoring and conservation of agents.

Table 2. Biocontrol status of the 25 highest-ranked alien invasive weed species, based on estimates of the total area invaded (Versfeld *et al.*, 1998), in South Africa. See Table 1 for definitions of control and implementation stages.

Weed species	Total invaded area (ha)	Number of agents available	Biocontrol status (stage of implementation)
<i>Melia azedarach</i> * ⁱ	3,039,002	—	Not yet targeted
<i>Pinus</i> spp. * ^h	2,953,529	—	Under investigation
<i>Acacia mearnsii</i> * ^h	2,477,278	1 (insect)	Unknown (Stage 2)
<i>Eucalyptus</i> spp. * ^h	2,429,329	—	Not yet targeted
<i>Lantana camara</i>	2,235,395	6 (insects)	Negligible (Stages 1 & 2)
<i>Acacia cyclops</i>	1,855,792	1 (insect)	Substantial (Stage 2)
<i>Acacia saligna</i>	1,852,155	1 (pathogen)	Complete (Stage 3)
<i>Opuntia</i> spp. * ^d	1,816,714	8 (insects)	Substantial (Stages 2 & 3)
<i>Jacaranda mimosifolia</i> * ^a	1,819,008	—	Not yet targeted
<i>Prosopis</i> spp. * ^c	1,809,229	2 (insects)	Negligible: new agents needed
<i>Solanum mauritianum</i>	1,760,978	1 (insect)	Unknown (Stage 1)
<i>Sesbania punicea</i>	1,404,505	3 (insects)	Complete (Stages 2 & 3)
<i>Caesalpinia decapetala</i>	1,317,243	1 (insect)	Unknown (Stage 1)
<i>Populus</i> spp.	1,305,019	—	Not yet targeted
<i>Acacia melanoxylon</i> * ^h	1,201,417	1 (insect)	Substantial (Stage 2)
<i>Ricinus communis</i>	1,194,142	—	Not yet targeted
<i>Morus alba</i> * ^d	997,960	—	Not yet targeted
<i>Psidium guajava</i> * ^d	759,844	—	Not yet targeted
<i>Cereus</i> spp.	745,688	2 (insects)	Unknown (Stage 2)
<i>Hakea</i> spp.	723,449	2 (insects)	Substantial (Stages 2 & 3)
<i>Eichhornia crassipes</i>	676,518	6 (4 insects, 1 mite & 1 pathogen)	Substantial (Stages 2 & 3)
<i>Rubus</i> spp. * ^d	647,347	—	Unsuccessfully targeted
<i>Acacia dealbata</i>	615,171	1 (insect)	Unknown (Stage 2)
<i>Agave</i> spp.	603,628	—	Not yet targeted
<i>Chromolaena odorata</i>	534,655	1 (insect)	Unknown (Stage 1)

* Beneficial species used for ornamental purposes^a, commercial forestry^h, agroforestry^c and fruit production^d.

IMPLEMENTATION OF BIOLOGICAL CONTROL

In essence, implementation involves the propagation and release of biological control agents, as well as sustained monitoring of their establishment and impact. In South Africa, these functions were previously the sole responsibility of biocontrol practitioners of the Plant Protection Research Institute (PPRI) (propagation and release of biocontrol agents) and of university-based researchers (post-release monitoring). However, biocontrol practitioners have several research responsibilities and, therefore, are disinclined to assume the practical burden of propagating sufficient agents to satisfy the demands of landowners. Consequently, one of the key elements of the NBCIS is the delegation of this function to

other State and private organizations. Besides the benefit of utilizing the agents far better, by substantially augmenting their populations and increasing their distribution in the field, researchers will now be able to focus more on the development of new strategies (e.g. optimal release methods) or new agents and on post-release evaluations. The NBCIS has identified several essential components in the implementation programme and these are discussed below.

Mass production and release of agents

The establishment of mass-rearing centres (MRCs) in targeted or appropriate areas will facilitate the establishment of large populations of biocontrol agents in the field and also ensure a constant supply of agents to various end-users. These centres are to be established in specifically targeted provinces or regions, and will be constructed according to the types of agents to be reared. Existing MRCs comprise either enclosed laboratories with controlled conditions or large outdoor tunnels or cages, usually covered by shade cloth. The MRC staff should include a manager, with horticultural and (preferably) entomological training, as well as several unskilled workers who will rear the agents.

The MRCs are funded, managed and staffed largely by the WFW Programme, although smaller-scale operations have been set up by private organizations (e.g. SAPPI Forests) or by individual landowners that have formed partnerships with implementing agencies. All MRCs have been established according to guidelines provided by researchers from the PPRI. Agents targeted by these operations are mostly new species that have not become widely established (Tables 1 & 2). So far only one MRC is fully operational, namely that of the WFW Programme based at Tzaneen (Northern Province) where agents for the weeds *Caesalpinia decapetala*, *Chromolaena odorata*, *Lantana camara* and *Solanum mauritianum* are currently being reared. The potential output of these MRCs is considerable, as has already been demonstrated at Tzaneen where in excess of 100,000 caterpillars of the defoliating moth *Pareuchaetes pseudoinsulata* (family Arctiidae) have been produced monthly for release against *Chromolaena odorata*. Recently, three smaller-scale MRCs have been established by SAPPI Forests in the provinces of KwaZulu-Natal and Mpumalanga, where new agents for use against *C. decapetala*, *L. camara* and *S. mauritianum* and are being reared. With financial support from the WFW Programme, these operations may be expanded to assume the role of fully-fledged MRCs that are able to supply agents to various end-users.

Collection and redistribution of established agents

Although, in previous years, agents have been established on several weeds, with considerable success in many cases (Tables 1 & 2), isolated weed populations can cause resurgences if they are not colonized by the agents. Consequently, the efficacy of established agents can be enhanced by collecting and releasing them in areas to which they have not yet dispersed or have been decimated by other factors (e.g. clearing or herbicidal operations, fires and flooding). Furthermore, certain agents are poor candidates for mass-rearing because of prolonged life cycles or difficulties in culturing, and these are best redistributed from established field populations. Examples of the latter included the complex of seed-feeding weevils (*Melantarius* spp.) (family Curculionidae) which attack

several species of invasive Australian *Acacia*, as well as the seed-feeding weevil *Erytemna consputa* (family Curculionidae) and moth *Carposina autologa* (family Carposinidae) on *Hakea sericea*. To facilitate redistribution, demarcated 'nursery' sites, which may be the original release sites, need to be established in safe areas that are less prone to disturbance (e.g. seasonal fires).

Agents whose efficacy can be advanced substantially by redistribution include those with poor dispersive abilities, such as the complex of cochineal insects (*Dactylopius* spp.) (family Dactylopiidae) that have been very successful against several invasive *Opuntia* cacti (Table 1). Also, in some cases where more than one species of agent is involved, the efficacy of biocontrol can be increased by ensuring that all agent species are present in weed infestations. For example, biocontrol of *Sesbania punicea* is maximized when all three agents are present (Hoffmann & Moran 1999), but only two agents have become widespread in South Africa whereas the third remains relatively localized.

Thus, the redistribution of established agents may be as important as the mass-rearing and release of new agents. The establishment of regional field implementation units (FIUs), comprising Implementation Officers that are detached from the MRCs, for the purposes of redistribution is intended to separate this function from that of mass production. The FIUs will also be responsible for other functions (see below).

Monitoring of establishment and assessments of impact

One of the main tasks of the FIUs will be to monitor the establishment of biocontrol agents that have been released or redistributed (Tables 1 & 2). Basic monitoring of the agents' density and distribution, in relation to regional weed infestations, will facilitate the selection of sites in specifically targeted areas for release or redistribution. This information will be entered into an extensive database, accessible to both weed managers and researchers, which can then be used to determine the need for further releases and to improve release strategies. The FIUs will also be responsible for the protection of the release sites from clearing operations, over-exploitation and other potential hazards (see below).

Primarily, post-release evaluations of the agents' impact on the weed populations will be the responsibility of the researchers, although Implementation Officers of the FIUs can also participate in the process. These studies will provide essential information on how the agents should best be integrated with existing control methods, so as to maximize their impact on the weeds. In addition, post-release evaluations can indicate which types of new agents should be prioritized for importation and research.

National co-ordination of the implementation strategy

A National Co-ordinator currently provides the link between funding bodies (e.g. WFW Programme), research organizations (e.g. PPRI and universities) and the various implementation organizations. In future, inter-agency liaison is likely to be delegated to organizations participating at a regional level, with information forwarded to the WFW Programme via the National Co-ordinator. A standardized reporting system and database will be developed to ensure that data pertaining to mass production, releases, redistribution

and monitoring can be accessed at a national level. A WFW Project Review Panel, comprising scientists from the WFW Programme, PPRI and other external organizations, has already been convened to assess progress on current biocontrol research projects, to identify research priorities and to facilitate the integration of biocontrol into weed management programmes.

CHALLENGES FACING IMPLEMENTATION OF BIOCONTROL

Although the NBCIS should ensure that biocontrol agents are produced and distributed in numbers that have never been achieved before, with the potential to substantially reduce the time required to bring weeds under biological control, there are several issues that need to be addressed in order to maximize the efficacy of the initiative.

Training and technology transfer

The WFW Programme is primarily a social upliftment initiative which, besides being aimed at improving water yields in impoverished areas, is also geared towards the creation of employment opportunities for semi-skilled and unskilled workers. As a result, personnel responsible for many of the MRCs or FIUs will tend to have limited botanical, and no entomological, experience. Indeed, practical problems in the propagation of healthy food plants, culturing of certain agents (e.g. disease outbreaks), release of agents (e.g. insufficient numbers at inadequate sites) and record keeping (e.g. lack of release records) have already been experienced. These problems are less severe where private organizations (e.g. SAPPI Forests), which mostly employ better-qualified staff, are involved with implementation.

To alleviate these problems, it is essential that basic training in horticulture, insect rearing and release techniques be provided to MRC managers and staff. In addition, a consultancy network which links researchers and MRC managers should be established to deal with *ad hoc* queries and problems and to provide the managers with updated information. In particular, researchers need to develop informative dossiers on each biocontrol agent that has been earmarked for mass production or redistribution. These dossiers should include information sheets containing life history details, guidelines for propagation and releases, and data sheets where information pertaining to releases and subsequent monitoring should be recorded. A national databank, like the Southern African Plant Invaders Atlas (SAPIA) (Henderson, 1999), should be configured to facilitate the mapping of sites where agents have been released or have become established.

Appropriate integration of control methods

Because the WFW Programme is focused primarily on mechanical and chemical control methods, careful planning is required to prevent these from interfering with biological control. Indeed, there have been instances in South Africa where conventional control methods have been antagonistic to the action of well-established biocontrol agents (Olckers *et al.*, 1998).

In some cases, populations of agents, notably those with poor dispersive abilities, may be decimated because the death of their host plants precedes their dispersive phase. The sessile cochineal insects (*Dactylopius* spp.) which are very effective agents of different *Opuntia* cacti and which depend on mobile first-instar nymphs ('crawlers') for dispersal, are particularly vulnerable to herbicide applications which kill the plants before the 'crawlers' develop. In these situations, chemical control should be restricted to uncontaminated (i.e. cochineal free) and isolated cactus populations where the risk of resurgence and further invasion is high. However, land managers have been reluctant to exclude contaminated plants from spraying operations, as evidenced by the widespread decimation of *Dactylopius austrinus* populations by a State-subsidized chemical control programme launched against jointed cactus, *Opuntia aurantiaca*, in the 1970s and 1980s (Moran & Zimmermann, 1991).

In other cases, herbicides, or the carriers and adjuvants with which they are used, may be detrimental to insect agents, either because of toxicity or because they cause suffocation by blocking the insects' spiracles. Although there are few reported cases of this, negative effects of herbicides have been recorded on insect and mite agents of water hyacinth, *Eichhornia crassipes* (Olckers *et al.*, 1998; Hill & Cilliers, 1999). In another example, unnecessary applications of herbicides on *Acacia saligna* trees that are infested by the rust fungus *Uromyces saligna* have proved detrimental because the chemicals destroy the spores that are needed to re-infect re-growth on the same plants (Zimmermann & Neser, 1999). The demarcation of 'no spray' zones and selection of 'insect friendly' herbicides or mycoherbicides that have minimal or no adverse effects on the agents are strategies that can enhance biocontrol considerably.

Conservation of biocontrol agents

The sustainability of biological weed control is dependent on the presence of the agents throughout the weeds' distribution. However, agents are often displaced or have become locally extinct where large infestations are cleared mechanically or treated chemically (see above) or where catastrophic events, such as flooding or severe fires, have occurred. The demarcation and preservation of patches of weeds to serve as 'agent reserves' within infestations will enable the agents to survive within an area and to re-colonize re-growth of the weed (Zimmermann & Neser, 1999). Some of these 'reserves' can also serve as 'nursery' sites for redistribution of the agents to new areas.

This procedure is critical for certain types of insect agents, notably seed-feeding species which cannot propagate on seedlings or re-growth but require older, reproductive plants for populations to persist (e.g. on *Hakea sericea*) (Gordon, 1999). Protected 'reserves' have also been advocated for most water weeds, where periodic flooding has limited the proliferation of the agents (Hill & Cilliers, 1999). However, this concept has met with some opposition because many land managers are convinced that the 'reserves' will become sources of re-infestation, which render the clearing operations ineffective in the long-term. Consequently, the well-intended actions of the WFW Programme could cause considerable harm to some of South Africa's most successful biocontrol programmes. Therefore, it is crucial that this concept becomes more widely accepted and that the 'reserves' are clearly marked in the field and accurately reflected in the appropriate maps and databases of the WFW Programme.

Preemptive programmes

Although many of South Africa's worst weed invaders have been subjected to biological control (Table 2), new and potentially invasive alien plant species continue to be introduced. Some are likely to become more problematic with time and may even replace weeds that have been brought under biocontrol as the main invasive species. Indeed, some species that currently have minor status in South Africa have already caused severe problems elsewhere in the world (e.g. *Mimosa pigra* and *Parthenium hysterophorus*), suggesting that their weed status may well increase. Other potential weeds have been deliberate introductions (e.g. the agroforestry tree *Leucaena leucocephala*) despite proven invasiveness in other countries. The implementation of biocontrol (e.g. establishing seed-feeding agents to limit seed dispersal) during the early phases of invasion can reduce the intensity of future control efforts and the time taken to achieve success.

The benefits of preemptive action were demonstrated in South Africa in 1960, with the campaign against *Hypericum perforatum*. The weed was already under biocontrol in North America and Australia when it began to invade natural ecosystems in South Africa. The establishment of two biocontrol agents has ensured that the weed has remained an insignificant invader in a few localized areas (Gordon & Kluge, 1991). Other recent preemptive programmes have involved the release of agents against *Leucaena leucocephala* and *Macfadyena unguis-cati* (Table 1), both of which are emerging as important invaders of the future (Zimmermann & Naser, 1999). However, as with most preemptive programmes, both of these projects have been opportunistic and poorly funded as it has been difficult to secure funding for low-priority weeds.

The prediction of future invasions by 'emerging' weeds, using existing databases that monitor the spread of alien species (e.g. SAPIA), will advance the case for preemptive programmes (Zimmermann & Naser, 1999). The challenge is to ensure that this procedure becomes more widely accepted and more amenable to funding, given that the savings in terms of later implementation could be considerable.

CONCLUSIONS

Although biological control of weeds is well established in South Africa, the concept of multi-organizational implementation is new. Although this concept is still in the early stages of development, the potential benefits for biological and integrated control of weeds are considerable, provided that the challenges are addressed. Indeed, the notion that biocontrol rarely solves alien plant problems in isolation, but that it can result in considerable savings in the cost of conventional control methods, justifies the objectives of the NBCIS. Co-ordination at both national and regional levels will be of prime importance in ensuring the transfer of up-to-date biocontrol technology to the implementing organizations and in determining policy that will prevent a recurrence of problems that have already been experienced. Ultimately, the success of this venture in South Africa will depend on the degree to which the integrated control procedures adopted by the WFW Programme will promote and conserve the biocontrol agents in which considerable efforts and finance have already been invested.

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